IN-SILICO APPROACH FOR SCREENING SEVERAL INHIBITORY PHYTO-CHEMICALS AND PEPTIDE BASED VACCINATION AGAINST DENGUE AND CHIKUNGUNYA VIRUSES

Submitted by

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Abstract

Dengue virus (DENV) and Chikungunya virus (CHIKV) both are *Aedes* mosquito-borne viruses which cause Dengue and Chikungunya diseases respectively. DENV is an arbovirus under Flaviviridae family and CHIKV is an alphavirus under Togaviridae family. *Aedes albopictus* and *Aedes aegypti* are two biological vectors, transmit DENV and CHIKV into the human body through biting. Epidemically Dengue and Chikungunya prove their fatalness over tropical and subtropical countries in the globe. It has been reported that about 400 million dengue cases and 22,000 deaths occur annually worldwide. DENV has four serotypes i.e., DENV-1, DENV-2, DENV-3 and DENV- 4. As per ECDC (2024), ~4,80,000 CHIKV infected human being existed across Asia, America, Europe and Africa. In 2024, more or less 200 deaths occurred globally. Genetic variations in envelope glycoprotein of DENV and CHIKV affect both clinical symptoms and infectivity. Although dengue and chikungunya show critical clinical symptoms and many troubles, there are still no effective vaccines or treatments available in the marketplace. Present effective vaccines against dengue are Dengvaxia and Qdenga. IXCHIQ is an effective vaccine against chikungunya. But each has some limitations. As for example, well famous Dengue vaccine Dengvaxia is applied for only 9-45 aged people and Qdenga for 6-16 aged children. These vaccines are unable to inhibit all DENV serotypes also. Chikungunya vaccine IXCHIQ is recommended only for adults (above 18 years old). Recently it has been reported that peptide-based vaccine may reduce other vaccines' limitations because this vaccine is cost-friendly, time saving, user-friendly, safe and secure. By modifying structure of peptide, multi-epitope structures can be preferred and designed against DENV and CHIKV. Through *in-silico* approaches, detailed analysis on different amino acids ranges of envelope protein like mutational study, epitope prediction, active site prediction, model creation, analysis of physico-chemical property, allergenicity, antigenicity, toxicity, immunogenicity, proteolytic cleavage site, BLASTp, ADMET analysis, molecular docking and simulation will be done for DENV and CHIKV. On the basis of these parameters, a novel peptide-based vaccine candidate may be designed which supposed to combat all the DENV serotypes and two CHIKV inhibiting novel peptide-based vaccine candidates may also be designed. UNIPROT, ClustalW, IEDB, CASTp, SWISS-MODEL, AlphaFold server, Expasy ProtParam tool, Allertop, Vaxijen, ToxinPred server, Expasy peptide

cutter tool, BLAST, ADMET, HDOCK server and GROMACS were utilized to perform specific functions related to above mentioned parameters. Envelope (E) glycoprotein sequences of DENV (390 seq_s for DENV-1, 418 seq_s for DENV-2, 400 seq_s for DENV-3 and 410 seq_s for DENV-4), E1 and E2 glycoproteins of CHIKV (350 seq_s for E1, 280 seq_s for E2) were analyzed. Length of the protein sequences of all DENV serotypes were 400 ± 100 amino acids. Length of E1 glycoproteins of CHIKV were 400 ± 100 amino acids and length of E2 proteins of CHIKV were 421 ± 2 amino acids. On the basis of literature review, specific ranges of mutational hotspots for DENV and CHIKV were considered for further analysis respectively. For DENV the ranges were 45-65, 110-140, 190-225, 255-290, 300-345 and 346-390. For CHIKV E1 protein conserved regions were 35-70, 113-130,164-188, 190-210, 227-249 and CHIKV E2 protein conserved regions were 43-60, 97-120 and 169-206. After molecular docking and simulation, finally peptide of strain A0A0D4D8J0 (45-65 range) of North America for DENV E protein, peptide of CHIKV E1 strain A0A343YNF1 (190-210 range) and peptide of CHIKV E2 strain F8QQQ6 (169-206 range) of Asia were selected to design stable and effective novel peptide-based vaccine candidates against DENV and CHIKV respectively.

Another approach of my *in-silico* work was to select phyto-chemicals (plant-derived bioactive compounds) to inhibit DENV and CHIKV. 71 phyto-chemicals against DENV and 69 phyto-chemicals against CHIKV were preferred for ADMET study. Drug-likeness characteristics of these phyto-chemicals were analyzed on the basis of Lipinski's rule of Five. With the help of ADMET analysis and drug-likeness properties, phyto-chemicals (Genistein, Baicalein, Quercetin and Artecanin) for DENV and phyto-chemicals (Genistein, Baicalein, Resveratrol, Epicatechin and Quercetin) for CHIKV were finally considered for molecular docking. PUBCHEM database, ADMET, AutoDock, GROMACS were utilized to perform specific functions. Among all phyto-chemicals, only Genistein exhibited maximum binding energy for DENV (ΔG = -6.06±1.1kcal/mol) E protein with different mutational ranges and also for CHIKV for both E1 (ΔG = -8.3 kcal/mol) and E2 (ΔG = - 7.1 kcal/mol). Molecular dynamics (MD) simulations reveal that Genistein (ligand) forms a stable complex with DENV and CHIKV envelope protein (target protein). But *in vivo* along with *in vitro* validation should be conducted before go to the market.